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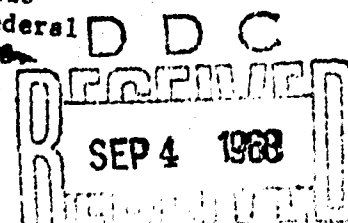
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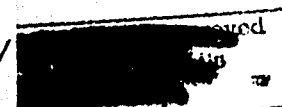
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THE INFLUENCE OF ASCORBIC ACID ON THE REDOX CAPACITY AND
THE DEVELOPMENT OF STREPTOMYCES GRISEUS CULTURES

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The basic premise for the functioning of a live organism is the corresponding Redox potential, E_h of the medium. But the chemical processes which bring this about show no uniform reaction in respect to the development of micro-organisms (6). One group of the Redox systems causes a total respiratory paralysis of the living cells. The watery solution of these systems produce a well defined E_h with a stability characterized by the fact that the O_2 or N -flow created no substantial change in the values of the potential (e.g. MoO_4^{4-}/Mn^{++} , $Fe/CN/^{4+}_6$, $Fe/CN/6^{3-}$). In the other group this stability is absent. The oxidized and reduced forms of the mainly organic Redox systems, which also exist in the metabolic products of living organisms, are not easily brought into equilibrium. These systems are therefore not electron active (4) and do not develop a well-defined electrode-potential (1) on the platinum electrode. They, i.e. their watery solutions, produce through the N -flow a continuous fall in the potential. This decrease in the potential-values can be lowered and brought to the initial level by adding O_2 . This proves that in the solutions of such Redox systems it is not the organic Redox system but the amount of O_2 content present in the fluid stage, i.e. the partial pressure of oxygen, that is responsible for the created values (7). The developing of electrode-potential-values is all the more possible in view of the formation of E_h which can be explained not only in relation to the proportion of the Redox components, but a similar conclusion can be reached on considering the H -ion concentration and the partial oxygen pressure as a symptom of potential formation (5). In the same way it is possible to

express the value of E_h as a function of OH-concentration and the partial O_2 pressure (pO_2) as follows:

$$E = E_0 - 0.06 \log \frac{(OH^-)}{\sqrt[4]{pO_2}}$$

where E is the created electrode-potential and E_0 represents the value of the potential reaching its peak when the (OH) concentration equals the fourth radical of the partial O_2 -tension.

In biological systems this is a result of cellular respiration reducing the O_2 contents in the medium and consequently diminishing the electrode-potential which can be better observed in air-excluding systems. This action depends on the intensity of the respiration. The lowest potential-level that is reached is a special characteristic of the living cells in a biological system. This decrease in E_h does not happen in the presence of well-defined, stable Redox systems and proves that cellular respiration is impossible. The potential of the living cells is not determined by the less stable Redox systems which do not interfere with respiration in the presence of O_2 dissolved in the medium and, as a consequence the O_2 consumption is shown by a drop in the electrode-potential. A sharp distinction between the Redox systems is, however, impossible from the point of view of stability. There are many known Redox systems -- among them ascorbic acid -- which influence the electrode-potential-values but are nevertheless of definite importance in respect to the formation of potential-values of the partial O_2 tension. Experiments on the mechanism of action of such Redox systems are definitely indicated.

Methods of Investigation

The influence of ascorbic acid on E_h was examined on synthetic (10) media. The measurements with streptomyces griseus species resulted in serial trials in bottled cultures: 50 Erlenmeyer flasks of 100 ml capacity were each filled with 30 ml of the medium, stopped up with cotton and sterilized for ten minutes at $110^\circ C$ in the autoclave. Following that, after ensuring the sterility with ultraviolet light, 1 ml of ascorbic acid solution and 2 ml sterile water were put into each of 20 flasks, 3 ml of ascorbic acid solution into another 20 flasks, and 3 ml of sterile water into 10 flasks. Then the pH of the medium was uniformly established with 40% NaOH. The ascorbic acid solution contained 100 mg of ascorbic acid per ml. The media so prepared were inoculated with agar slope, rinsed test-tube cultures.

In order to be able to follow the dissolution of ascorbic acid in the solution, we have omitted to inoculate 10 ascorbic acid-containing media in each batch. The inoculation took place in thermostats at a temperature of $26^\circ C$.

The measurement of the electrode-potential of the media took place on one of the platinum electrodes immersed in the medium solution -- opposite a Calomel-saturated electrode (9). The determining of the ascorbic acid content was obtained through dichlorophenol-indophenol solution (3) titer. That of the Redox capacity of the cultures with the iodometric system. The K-I iodine solution oxydized or reduced in an acid environment, depending on the medium.

Evaluation of Results

The electrode-potential of the fluid of the micro-organism culture decreases by about 200 mV after the addition of ascorbic acid (Fig. 1, curve 1; Fig. 2, curves 1 and 2). This decrease in potential-values signifies that the free O_2 content of the medium is greatly diminished, because the ascorbic acid has oxydized irreversibly into dehydro-ascorbic acid. The free oxygen content of the medium can decrease as long as the electrode-potential level does not reach the value corresponding to the quotient of the ascorbic acid/dehydroascorbic acid. The experimental finding that these compensating potential-values of the culture are close together even with a high ascorbic acid content, is due to the above fact (Fig. 2, curve 1 and 2).

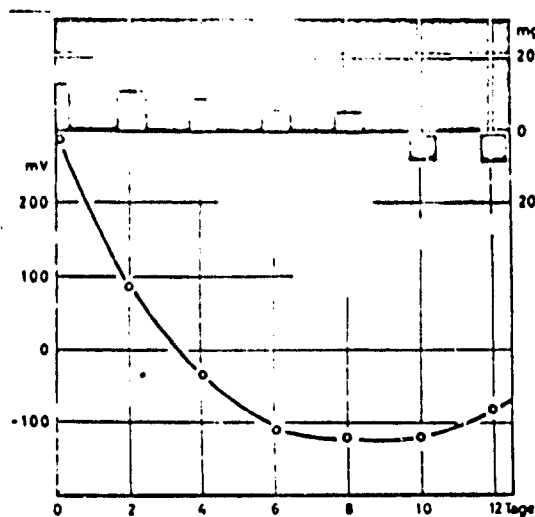


Fig. 1. The curve representing the consumption of oxygen by the streptomyces griseus culture and the Redox capacity. The light columns show the oxydations and the dark ones the reduction capacity of Iod-mg.

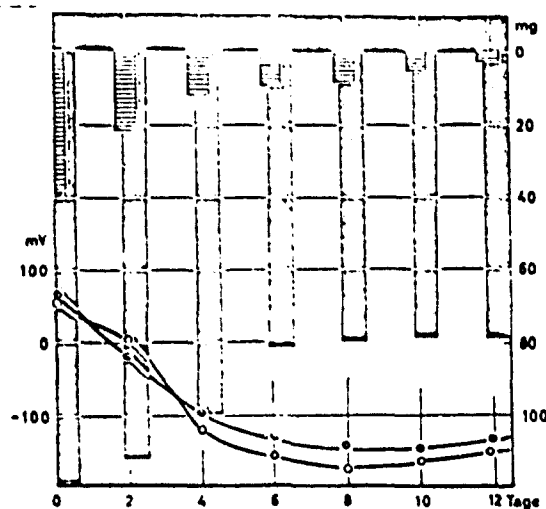


Fig. 2. The ascorbic acid induced change of the potential curve and the Rodox capacity of the streptomyces griseus culture. Curve 1 (o—o) and the shaded column represent the data of the 100 mg, and curve 2 (•—•) and the dark column the data of the 300 mg ascorbic acid containing culture.

Streptomyces griseus -- as an aerobic micro-organism -- has a greater oxygen requirement than an ascorbic acid containing medium can provide it with and therefore its development is greatly inhibited. The electrode-potential of the streptomyces griseus culture which we have used is, like that of the mold fungus, reduced after inoculation in a medium containing ascorbic acid as well as in one free of it. The reason for this is that in the more favorable media with a higher O_2 tension, the development of the micro-organisms is increased. This is expressed in the higher O_2 consumption. During the first three days a continuous mycelium membrane develops in the ascorbic acid-free cultures but not in the cultures containing ascorbic acid.

After the inoculation with micro-organisms, the medium consumption of oxygen is twofold: partly to provide the oxygen necessary for the development of the culture and partly as oxydation of the ascorbic acid; the replacement of O_2 occurs through diffusion from the air space. This diffusion is greatly reduced by the mycelium membrane which developed between the medium and the air space, thereby creating a pO_2 difference between the upper and lower layers of the medium. This explains the fact that the potential measurement value of electrodes placed in different depths of the cultures diminished from the surface towards the bottom (2, 8). The O_2 of the air has its greatest effect on the surface of the medium. As a result, the ascorbic acid on the surface is slightly oxydized and the ascorbic acid capacity of the system locally exhausted,

so that a development is here possible. To these circumstances can be ascribed the formation of a very thin mycelium layer in a comparatively rich ascorbic acid content, but there is no further possibility of growth.

Experiments with aerobic micro-organisms indicate that the oxydation of ascorbic acid in the cultures is hindered. We observed the retrogression of ascorbic acid content in inoculated and uninoculated media and found that the oxydation of the 100 mg of ascorbic acid contained in the uninoculated medium was completed within 5 days, while in the cultures after a period of 12 days 50% of the added ascorbic acid was evident. In the uninoculated media containing 300 mg of ascorbic acid there was no sign of ascorbic acid after ten days, while the cultures after 12 days still contained 20 mg of it (Fig. 3, curves 1a and 1b as well as 2a and 2b).

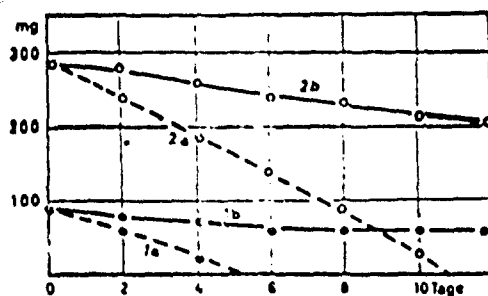


Fig. 3. Changes in the ascorbic acid contents in inoculated media (1a, 2a) and culture (1b, 2b).

The reducing ability of ascorbic acid as expressed by the Redox capacity, is not substantially influenced by the metabolic products of the culture. The initially slight oxydation ability of the medium diminished in the course of the development of the culture, in order to take on the reducing characteristic which is then retained also with an insignificant capacity value (Fig. 1). The Redox capacity of ascorbic acid-containing media is substantially greater (fig. 3), and therefore the changes observed after inoculation are conditioned practically by the reduction of ascorbic acid.

Summary

Experiments with *Streptomyces griseus* cultures showed that growth of the aerobic micro-organisms is inhibited by ascorbic acid. Inhibition was found to be due to reduction in oxygen tension by the ascorbic acid. Double utilization of the oxygen dissolved in the medium -- for oxydation of the ascorbic acid and for respiration of the organisms -- causes a

reduction in rate of transformation of ascorbic acid into dehydroascorbic acid in inoculated media as compared to control media.

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